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# Genetic Relationships of Some Barley Cultivars, Based on Morphological Criteria and RAPD Fingerprinting

[1]Hanaa H. El-Shazly and [2]Zainab El-Mutairi
[1]Department of Biological Sciences and Geology, Faculty of Education,
Ain Shams University, Cairo, Egypt
[2]Department of Biology, Girls College of Education, Al-Kharg, Saudia Arabia

**Abstract:** In this study, the relationship of ten Saudi Arabian local and imported barley cultivars, based on evidences derived from agro-morphological traits and RAPD fingerprinting. Morphological measurements revealed that the two Egyptian cultivars, Giza 123 and Sahrawy showed good vegetative growth and yield productivity. In addition, morphological criteria of some cultivars developed by King Saud University reflected the morphological features and yield potential of their parents; the Egyptian cultivars Giza 121, Giza 123 and the American cultivar Gustoe. Four out of 11 primers gave a total of 53 bands in RAPD reactions, 36 of which were polymorphic. The number of bands ranged from 11 to 18 per primer and between 22 and 28 per cultivar with size range from 275 to 2072 bp. The analysis of RAPD profiles produced some cultivar-specific bands; these include the presence of unique bands in cv Giza 123, cv Local and cv Gustoe and cv Sahrawy and the absence of one band in cv Giza 121, cv Giza 123, cv Giza 128 and cv Sahrawy. Data were analysed using NTSYS-pc software and genetic distance trees were produced by the UPGMA and the Neighbor joining (NJ) methods. The trees based on morphological data reflected the pedigree of some cultivars, in particular the close relationships between cultivars developed by King Saud University and their parents. However, in the trees based on RAPD data, the genetic relationships among the studied cultivars were not in full agreement with their pedigree and showed resemblance between morphologically different cultivars. In the trees based on the analysis of the combined morphological and RAPD data, the grouping of some of the six rowed cultivars was in agreement with their pedigree, specially the two cultivars Giza 121 and KSU 103 and the two cultivars Giza 123 and KSU 102.

**Key words:** Barley cultivars, DNA fingerprints, RAPD-PCR poly morphism, morphological criteria

## INTRODUCTION

In recent year, molecular evidences derived from DNA using electrophoretic techniques have provided powerful markers for the study of several aspects of biology. Currently, the technique of choice is the RAPD markers (Randomly Amplified Polymorphic DNA). This approach is based on using the Polymerase Chain Reaction (PCR) as proposed by Williams *et al.* (1990) to amplify DNA sequences with a single short (9-10 bp) primers of arbitrary nucleotide sequence. It requires small amounts of DNA, easy to perform and reveals dominant molecular markers of ultimate potentialities in several fields of plant science including systematics and evolution (Witkus *et al.* 1994), gene mapping (Barua *et al.*, 1993; Komatsuda *et al.*, 1997) and genetic diversity (Bernard *et al.*, 1997; Mohamed, 2004). RAPD markers have been used to determine genetic relationships in several plant species; examples include flax (Fu *et al.*, 2001; Diederichsen and Fu, 2001), sorghum (Dahlberg *et al.*, 2002), date palm (Soliman *et al.*, 2003) and between species in some genera such as *Morus* (Awasthi *et al.*, 2004) and *Vigna* (El-Kholy, 2005)

The genetic diversity in barley and the relationship of barley cultivars have been addressed by using biochemical evidence derived by the electrophoretic separation of the seed protein hordein (Faccioli *et al.*, 1999) and different isozymes (Volis *et al.*, 2001). Evidence obtained from electrophoretic separation of the seed protein hordein was also combined with morphological criteria to investigate genetic diversity in 49 accessions of naked barley (Atanassov *et al.*, 2001). Morphological criteria were also combined with the DNA fingerprinting methodology AFLP (Amplified fragment length polymorphism) to illustrate the genetic relationships of 30 cultivars of spring two-rowed and six-rowed barley from Europe and America (Schut *et al.*, 1997) and to finger print 15 cultivars of Egyptian barley (El-Rabey *et al.*, 2002).

**Corresponding Author:** Hanaa H. El-Shazly, Department of Botany, Girls College of Education, Science Departments, Bureida, Saudi Arabia

RAPD markers have been widely used to study genetic diversity in barley in combination with other molecular markers (Russell *et al*., 1997; Fernândez *et al*., 2002). Examples of the use of RAPD markers to study genetic aspects of barley also include the study of Bernard *et al*. (1997) on the genetic diversity among wild barley (*Hordeum spontaneum*) in the near east and the investigation by Hang *et al*. (2000) on 16 cultivars of two-rowed and six-rowed barley used for beer production in North America. Similar studied utilizing RAPD markers have been carried out on barley from Syria (Showman *et al*., 2001), the spring barley in Europe (Kuczynska *et al*., 2001) and the hullness barley from the Tibet region (Yu *et al*., 2002).

Barley is one of the most important fodder and food crops in the Kingdom of Saudia Arabia. Recently however, the cultivation of barley has declined due to the management measures on the consumption of water in the Kingdom. It is therefore desirable to develop cultivars of high yield that can withstand the dry conditions prevailing in most parts of the country. For this purpose investigating the genetic fingerprinting of the currently used barley cultivars is of prime importance. The aim of the present study is to define genetic fingerprints for different cultivars of barley cultivated in Saudi Arabia and determine their relationships, based on morphological criteria and DNA fingerprints as revealed by RAPD-PCR polymorphism.

## MATERIALS AND METHODS

**Plant material:** Grain samples of ten cultivars of barley currently cultivated in Saudia Arabia have been secured from different sources; they include four Egyptian cultivars from the Agricultural Research Center (ARC) in Giza, Egypt, three breeding lines developed by King Saud University in Riyadh, Saudi Arabia, one Saudi land race called cv Local, one cultivar from ICARDA (International Center for Agricultural Research in Dry Lands) in Aleppo, Syria and one American cultivar that is currently widely cultivated in Saudia Arabia. The names of these cultivars and their sources and the pedigree for six of them are given in Table 1. For the measurements of morphological characters, plants of all cultivars were grown from November 2003 to April 2004 in the Dirab farm of King Saud University in Riyadh under natural conditions in randomized blocks, as plants grow, irrigation and fertilizers were supplied as recommended in barley farming practices. At maturity 12 quantitative morphological characters were measured for ten plants for each cultivar and the state of three qualitative criteria was determined. The examined traits are shown in Table 2.

The RAPD fingerprinting experiments were performed in the Research Center of King Faisal Specialist Hospital in Riyadh. Grains of all cultivars were germinated in small pots in farm soil at a temperature range of 16 to 25°C. After ten days of germination fresh leaves of seedlings were harvested and immediately frozen in liquid nitrogen and kept at -70°C until use. For DNA extraction, leaf samples were powdered in liquid nitrogen and DNA extracted using the DNA easy Plant Mini Kit from Qiagen as recommended by the manufacturer. RNA was removed by RNase and proteins and carbohydrates were removed by precipitation using acetic acid and DNA was purified by precipitation in a solution of guanidine hydrochloride and ethanol. The DNA pellet was then washed twice in ethanol, dried and dissolved in TE buffer.

RAPD fingerprinting was performed using each of 11 primers (Proligo) in 25 μL reaction volume containing the following reagents: 2.0 μL of dNTPs (2.5 mM), 1.5 μL of $MgCl_2$ (25 mM), 2.5 μL of 10x buffer 2.0 μL of primer, 2.0 μL of template DNA (50 ng $\mu L^{-1}$), 0.3 μL of Taq polymerase (5 U $\mu L^{-1}$) and 14.7 μL of sterile dd $H_2O$. Amplification was carried out in 2400 Perkin Elmer Gene Amp PCR thermocycler as follows: 94°C for 4 min followed by 40 cycles at 94°C for one min; 37°C for one min. and 72°C for two min. The reaction was finally incubated at 72°C for 10 min. The RAPD products were electrophorased in 1.4% agarose gel in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA pH = 8). The run was performed at 100 volt for 60 min. The gels were stained in 0.2 μg $mL^{-1}$ ethiditim bromide and photographed using gel documentation system (Gel Doc BioRad, 2000) under UV transilliuminator. Each experiment was repeated twice and only stable products were scored.

**Data scoring and analysis:** The 15 examined morphological traits are listed in Table 2. The relationship among the examined cultivars was estimated based on differences among them in both morphological traits and RAPD fingerprinting separately and in combination. The quantitative morphological traits were given codes ranging between 0 and 3 depending on the variation in the average value for the measured traits. The qualitative traits on the other hand were all two-state characters and were coded as 0 or 1. For the analysis of RAPD data, the DNA bands in the RAPD profiles were scored in binary matrices, where 0 stands for the absence and 1 stands for the presence of bands in each individual sample.

The relationship between the examined cultivars was estimated using the Dice coefficient of similarity (Dice, 1945). The Dice equation is included in the computer program NTSYS-pc (Rohlf, 1993), which has been used for data analysis. Construction of distance trees illustrating the relationships among the studied cultivars was performed using the unweighted pair group method using arithmetic average (UPGMA) proposed by Sokal and Michener (1958) and the Neighbor joining (NJ) method (Saitou and Nei, 1987); both methods are also as implemented in the NTSYS-pc program.

## RESULTS

**Measurements and description of morphological characters:** The measurements of the 12 quantitative morphological characters±standard deviation (SD) are given in Table 2. Plant height ranged between 84.7±6.67 cm and 48.7±2.91 cm. The two-rowed cultivars cv Local and cv Giza 128 were significantly taller than the six-rowed cultivars. Of these cultivars; cv Sahrawy was the tallest, while cv Gustoe was the shortest. The value of flag leaf length ranged between 23.95±3.65 cm for cv Local and 16.95±1.61 for cv Sahrawy. High values for flag leaf width, on the other hand, were scored in cv Gustoe (1.44±0.32 cm) and cv Giza 123 (1.41±0.35 cm). The cv Local flowered much earlier than the other nine cultivars, it required only 61.25±2.06 days to heading; the two cultivars Gustoe and Sahrawy, on the other hand, required much longer time to heading (115.5±8.19 days for the former and 114.0±11.55 for the latter; the other seven cultivars needed about 100 days to heading (Table 2).

Six traits derived from the spike were measured for all cultivars (Table 1) and these traits are shown in Fig. 1 and measurements of these traits are given in Table 2. The longest spikes characterized the two-rowed cultivars Local (13.9±21.3 cm) and Giza 128 (11.58±1.03 cm) and the six-rowed cultivar Giza 123 (11.44±1.48 cm). The two-rowed cultivars are also characterized by thin spikes compared to the six-rowed cultivars (Table 2). Central spike awn length was highest in cv Local (16.51±1.84 cm) followed by cv Giza 128 (14.66±1.7 cm) and the two-six-rowed cultivars Giza 123 (14.59±1.67 cm) and KSU 102 (14.45±1.56 cm). Lateral spike awn length, on the other hand was taller in cv Giza 123 (16.51±1.84 cm) followed by cv KSU 102 (14.93±1.51 cm), cv Giza 121 (14.65±3.78 cm) and cv KSU 103 (14.22±2.32 cm); the lateral awn length is absent in the two-rowed cultivars (cv Local and cv Giza 128). Rachis was particularly tall in cv Giza 123 and cv Gustoe and short in cv Giza 121, cv KSU 102 and cv KSU 103 (Table 2). The Egyptian cv Giza 123 produced the highest number of grains per spike followed by cv KSU 102; the number of grains per spike was significantly low in the six-rowed cultivar Giza 121 and much lower in the two-rowed cultivars Local and Giza 128 (Table 1). The tallest grains were found in cv Local and cv KSU 103 and shortest grains in cv Gustoe, the grains were broader in the two-rowed cultivars than the six-rowed cultivars (Table 2).

Only three qualitative traits were examined in this study; these are, number of rows per spike, stem toughness and the erection of spike after ripening. The number of spike rows is two in the Saudi Local cultivar and the Egyptian cultivar Giza 128; these two rowed cultivars are also characterized by weak stem. The six-rowed cultivars have tough stem and the two-rowed cultivars have weak stem, meanwhile only cv Giza 128 has dropping spikes after ripening.

**Analysis of RAPD profiles:** Four primers produced stable and reliable bands in their RAPD profiles; the sequence of these primers and the number and types of the bands produced by each primer are given in Table 3. The four

Table 1: Names and sources of the barley cultivars used in this study and the pedigree for six of them

| Ser. No. | Cultivar | Source | Pedigree |
|---|---|---|---|
| 1 | Giza 121 | ARC - Egypt | Baladi 16 × Atsel |
| 2 | Giza 123 | ARC - Egypt | Giza 117 × FAO 36 |
| 3 | Rihane/Lignee | ICARDA-Syria | - |
| 4 | Local | Saudi Arabia | - |
| 5 | KSU. BL. 102 | King Saud Univ. | Giza 123 × C-C-89 |
| 6 | KSU. BL. 103 | King Saud Univ. | Giza 121 × Gustoe |
| 7 | KSU. BL. 104 | King Saud Univ. | Giza 123 × Gustoe |
| 8 | Gustoe | American | - |
| 9 | Giza 128 | ARC - Egypt | - |
| 10 | Sahrawy | ARC - Egypt | Baladi 16 × Gem |


Fig. 1: The spike features of the ten examined barley cultivars

Table 2: Average value ± SD of the measured quantitative morphological traits and the state of qualitative traits in the barley cultivars

| Cultivars<br>Trait | Giza 121<br>Mean±SD | Giza 123<br>Mean±SD | Rihane/<br>lignee<br>Mean±SD | Local<br>Mean±SD | KSU.<br>BL. 102<br>Mean±SD | KSU.<br>BL. 103<br>Mean±SD | KSU.<br>BL. 104<br>Mean±SD | Gustoe<br>Mean±SD | Giza 128<br>Mean±SD | Sahrawy<br>Mean±SD |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant height (cm) | 55.3 | 67.90 | 59.50 | 84.70 | 54.00 | 51.00 | 59.30 | 48.70 | 82.90 | 71.30 |
| | 4.90 | 9.39 | 8.21 | 6.67 | 10.79 | 8.39 | 8.22 | 2.91 | 3.81 | 5.66 |
| Flag leaf length (cm) | 17.26 | 21.57 | 13.85 | 23.95 | 19.30 | 18.40 | 20.41 | 18.69 | 18.92 | 16.95 |
| | 2.52 | 3.60 | 1.53 | 3.65 | 1.99 | 2.16 | 0.90 | 2.38 | 2.80 | 1.61 |
| Flag leaf width (cm) | 0.99 | 1.41 | 1.06 | 0.81 | 1.03 | 1.11 | 1.58 | 1.44 | 0.92 | 1.19 |
| | 0.11 | 0.35 | 0.17 | 0.16 | 0.16 | 0.09 | 0.19 | 0.32 | 0.14 | 0.15 |
| Days to heading | 102.8 | 102.0 | 99.00 | 61.25 | 99.00 | 100.0 | 106.8 | 114.0 | 95.75 | 115.5 |
| | 0.50 | 1.63 | 3.46 | 2.06 | 2.71 | 3.46 | 3.78 | 11.55 | 0.50 | 8.19 |
| Spike length (cm) | 7.78 | 11.44 | 9.72 | 13.92 | 10.06 | 9.89 | 9.81 | 8.45 | 11.58 | 9.87 |
| | 1.52 | 1.48 | 1.86 | 1.37 | 1.06 | 1.18 | 1.26 | 0.44 | 1.03 | 2.21 |
| Spike width (cm) | 1.01 | 1.11 | 1.09 | 0.75 | 1.09 | 1.10 | 1.09 | 0.88 | 0.80 | 1.04 |
| | 0.17 | 0.09 | 0.19 | 0.12 | 0.17 | 0.18 | 0.15 | 0.16 | 0.16 | 0.18 |
| Central awn length (cm) | 12.54 | 14.59 | 13.05 | 16.51 | 14.45 | 11.89 | 12.70 | 10.26 | 14.66 | 12.63 |
| | 4.13 | 1.67 | 0.50 | 1.84 | 1.56 | 2.11 | 1.18 | 1.03 | 1.70 | 1.28 |
| Lateral awn length (cm) | 14.65 | 16.67 | 14.13 | 0.00 | 14.93 | 14.22 | 13.65 | 11.89 | 0.00 | 13.80 |
| | 3.78 | 1.72 | 0.69 | 0.0 | 1.51 | 2.32 | 1.39 | 1.17 | 0.0 | 1.58 |
| Rachis length (cm) | 0.28 | 0.45 | 0.77 | 0.67 | 0.26 | 0.27 | 0.66 | 0.76 | 0.55 | 0.34 |
| | 0.05 | 0.36 | 1.33 | 0.16 | 0.05 | 0.09 | 0.58 | 1.01 | 0.19 | 0.10 |
| No. of grains/spike | 52.20 | 67.40 | 61.90 | 30.30 | 63.40 | 60.00 | 58.20 | 55.00 | 25.40 | 60.20 |
| | 10.60 | 6.80 | 15.09 | 3.50 | 9.14 | 21.04 | 12.98 | 9.58 | 3.27 | 14.71 |
| Grain length | 1.11 | 1.21 | 1.08 | 1.30 | 1.19 | 1.30 | 1.14 | 0.92 | 1.25 | 1.12 |
| | 0.11 | 0.13 | 0.10 | 0.16 | 0.11 | 0.09 | 0.14 | 0.08 | 0.09 | 0.05 |
| Grain width | 3.25 | 3.28 | 3.53 | 4.12 | 3.51 | 3.18 | 3.53 | 3.01 | 4.25 | 3.35 |
| | 0.43 | 0.38 | 0.40 | 0.54 | 0.46 | 0.31 | 0.51 | 0.22 | 0.22 | 0.39 |
| Spike rows | 6 | 6 | 6 | 2 | 6 | 6 | 6 | 6 | 2 | 6 |
| Stem toughness | Tough | Tough | Tough | Weak | Tough | Tough | Tough | Tough | Weak | Tough |
| Spike erection | Erect | Erect | Erect | Erect | Erect | Erect | Erect | Erect | Drop | Erect |

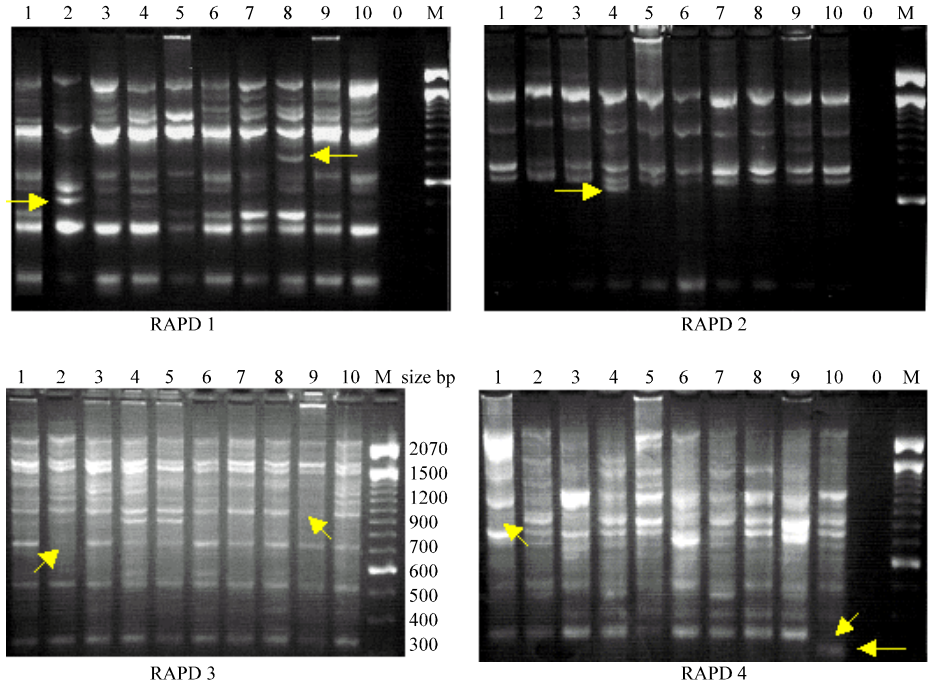


Fig. 2: The RAPD profiles produced by the four primers, which produced stable and reliable RAPD products in the genome of the studied barley cultivars, the cultivars are numbered as in Table 1. Horizontal arrows point to present unique bands and oblique arrows to absent unique bands

primers produced a total of 53 bands, ranging in size between 2072 and 275 bp, of these bands 17 are monomorphic and 36 are polymorphic, the latter type of bands include 28 true polymorphic bands that are present or absent in at least two cultivars and eight autapomorphic bands, which include four bands present in one cultivar and four bands found in all cultivars but absent in only one cultivar (Fig. 2 and Table 3).

Table 3: Nucleotide sequence of the four primers that produced stable and reliable RAPD products in the genome of the studied barley cultivars and the types and numbers of bands produced by each primer

| Primer | Nucleotide sequences | No. of bands | Polymorphic bands | Monomorphic bands | Autapomorphic (Unique) bands | Polymorphism(%) |
|---|---|---|---|---|---|---|
| RAPD 1 | 5' ACC CGA CCT G '3 | 13 | 9 | 4 | +2 | 69.23% |
| RAPD 2 | 5' AGA GCG TAC C '3 | 10 | 7 | 3 | +1 | 70.00% |
| RAPD 3 | 5' TGC CTT GCA C '3 | 16 | 11 | 5 | -2 | 68.75% |
| RAPD 4 | 5' GTC GAA CGA G '3 | 14 | 9 | 5 | +1-2 | 64.28% |
| Bands | | 53 | 36 | 17 | 8 | 67.92% |

Table 4: Types and numbers of bands produced by four primers in the studied cultivars of barley

| Band type | Giza 121 | Giza 123 | Rihane/ lignee | Local | KSU. BL. 102 | KSU. BL. 103 | KSU. BL. 104 | Gustoe | Giza 128 | Sahrawy |
|---|---|---|---|---|---|---|---|---|---|---|
| Polymorphic | 11 | 14 | 14 | 16 | 13 | 13 | 13 | 14 | 16 | 16 |
| Monomorphic | 11 | 10 | 11 | 11 | 11 | 11 | 11 | 10 | 11 | 11 |
| Unique bands present | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| Unique bands absent | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Total | 22 | 26 | 25 | 28 | 24 | 24 | 24 | 26 | 28 | 27 |

The RAPD banding profiles revealed by four primers are illustrated in Fig. 2. RAPD 1 produced 13 bands including four monomorphic and nine polymorphic, two of them are autapomorphic; one (500 bp) unique cv Giza 123 (2) and the other (750 bp) unique to cv Gustoe (8). RAPD 2 produced 10 bands comprised of three monomorphic bands and seven polymorphic that include one band (500 bp) unique to cv Local (3). The primer RAPD 3 produced 16 bands, five of them are monomorphic and 11 polymorphic including two bands that were found in nine cultivars but absent in one, these are a 700 bp band absent in cv Giza 123 (2) and a 950 bp band absent in cv Giza 128 (9). Primer RAPD 4 produced 14 bands five of which are monomorphic and nine polymorphic, these bands include one band (300 bp) unique to cv Sahrawy (Fig. 2). The banding profile of this primers also illustrates the absence of two unique bands, one 900 bp from the profile of cv Giza 128 and one (350 bp) from the profile of cv Sahrawy.

The number and types of RAPD bands revealed in the genomes of the studied barley cultivars are given in Table 4. The two-rowed cultivars Local and Giza 128 produced the highest number of total (28) and polymorphic (16) bands followed by the six-rowed cv Sahrawy with 27 total bands and 16 polymorphic bands. The number of total bands in the RAPD profile of the remaining cultivars ranges between 22 for cv Giza 121 and 26 for cv Giza 123 and cv Gustoe. Four unique bands were scored in the RAPD profiles of the ten examined cultivars; two in cv Gustoe and one in each of cv Giza 123 and cv Local, but no unique bands were detected in the genome of other cultivars. Two bands (700 and 950 bp) were absent in the RAPD 3 profile of cv Giza 123 and Giza 128, respectively.

**Relationships of barley cultivars:** The genetic distance trees produced by the UPGMA and NJ methods based on morphological data are shown in Fig. 3. In both trees close

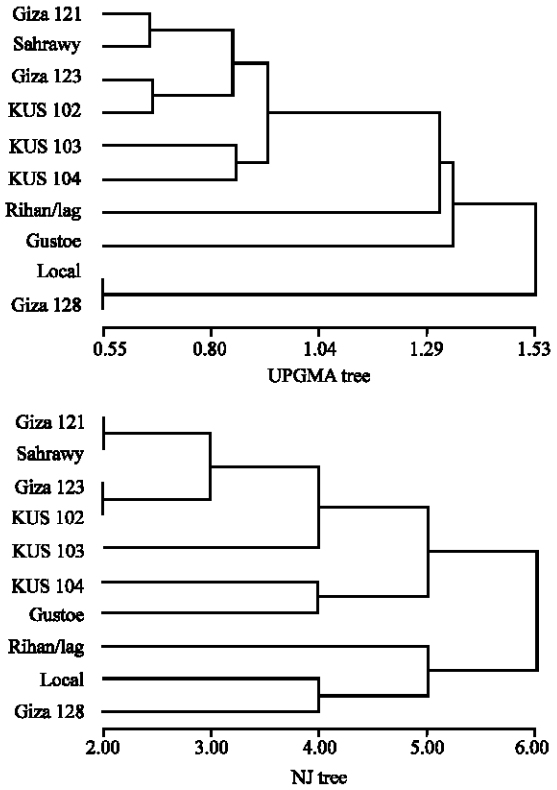


Fig. 3: The relationships of barley cultivars based on morphological data expressed as UPGMA tree and a NJ tree

relationships were found between the two-rowed spiked cultivars Local and Giza 128 that were grouped together and separated from the six-rowed cultivars. In the UPGMA tree, close relationships were also found between cv Giza 121 and cv Sahrawy, cv Giza 123 and KSU 102 and cv KSU 103 and cv KSU 104, respectively, these six cultivars together formed a major group. In this tree, the two cultivars Rihane/Lignee and Gustoe appeared distant from the other six-rowed cultivars and

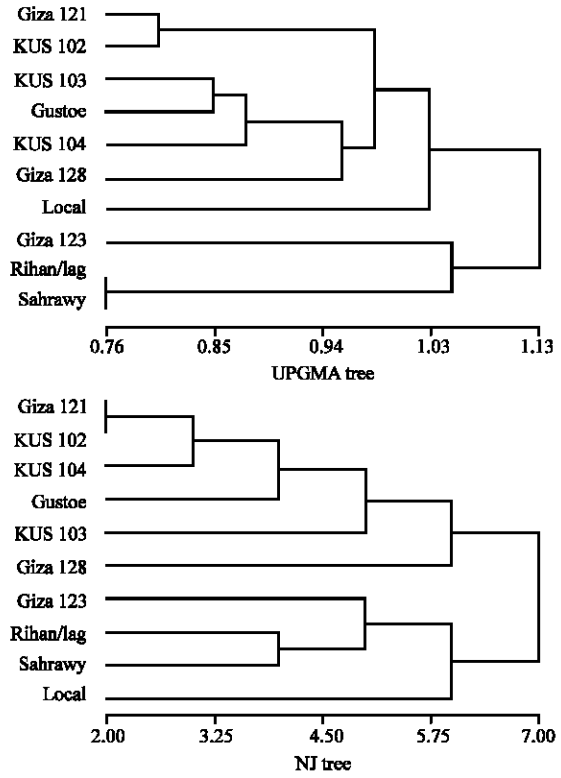


Fig. 4: The relationships of barley cultivars based on RAPD data expressed as UPGMA tree and a NJ tree

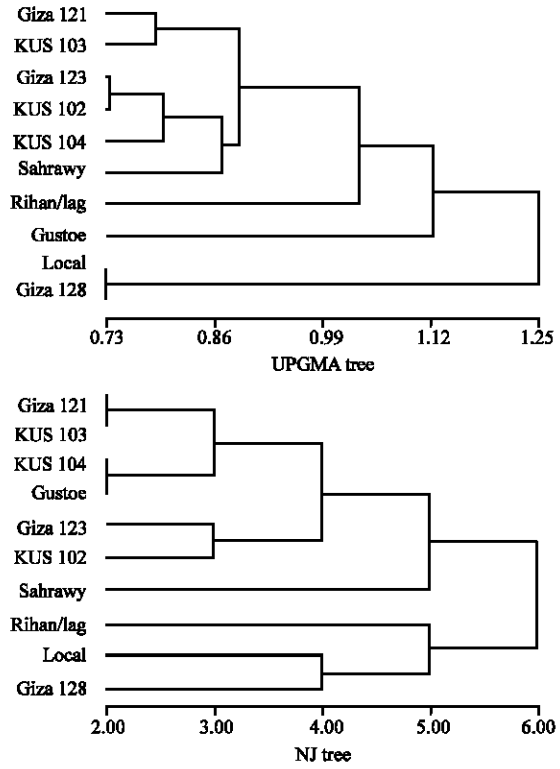


Fig. 5: The relationships of barley cultivars based on morphological traits and RAPD data expressed as UPGMA tree and a NJ tree

the two-rowed cultivars Giza 128 and Local. In the NJ tree the cv KSU 104 showed close relationship to cv Gustoe and cv KSU 103 showed close resemblance to the group comprised of Giza 121, cv Sahrawy, cv Giza 123 and cv KSU 102 (Fig. 3). Both UPGMA tree and the NJ tree reflect the pedigree of some of the studied cultivars, in particular, the close relationships between the cultivars developed by King Saud University and their parents.

The genetic distance trees produced by the UPGMA and NJ methods based on the analysis of RAPD data are presented in Fig. 4. In these trees the two-rowed cultivars were not grouped together although distinct from the six-rowed cultivars. In both UPGMA and NJ trees, the five six-rowed cultivars Giza 121, KSU 102, KSU 103, KSU 104 and Gustoe and the two-rowed cultivar Giza 128 from a major group, the cv Local was related to this group. The three six-rowed cultivars Giza 123 Sahrawy and Rihane/Lignee comprised a small group, in which the former cultivar is clearly separated from the latter two cultivars. In the NJ tree, however these three cultivars form one group to which cv Local was the closest cultivar, the other two-rowed cultivar (cv Giza 128) was closer to, but distinct from, the other five six-rowed cultivars.

The relationships of barley cultivars, based on the analysis of morphological traits and RAPD data combined by using the UPGMA and NJ methods are shown in Fig. 5. In these trees, the two cultivars with two-rowed spike were clustered together. Moreover, the grouping of some of the six rowed cultivars was in agreement with their pedigree, especially for the position of the two cultivars Giza 121 and KSU 103 and the two cultivars Giza 123 and KSU 102.

## DISCUSSION

The measurements of 15 agro-morphological traits of the ten studied barley cultivars clearly distinguished the two-rowed cultivars; the Saudi cv Local and the Egyptian cv Giza 128, from the eight six-rowed cultivars, not only in the qualitative characters but also in some quantitative characters. These two cultivars have taller plants longer and wider spikes, shorter time to heading and lower number of grains per spike. The early heading and fast vegetative growth are good criteria for use of these two cultivars as green fodder crops as proposed for cv Local by Haiba (1978) and Al-Dos *et al.* (2000).

The Egyptian six-rowed cultivar Giza 123 is distinguished from the other six-rowed cultivars by faster vegetative growth, longer spikes and higher number of grains per spike. This result agrees with the findings of Al-Dos *et al.* (2000) and confirms their recommendation for use of this cultivar as green fodder plant and for grain production. On the other hand, the short cultivar Gustoe that was reported by these authors to produce more grains compared to other cultivars including cv Giza 123 was not found as productive as cv Giza 123 and all other six-rowed cultivars except cv Giza 121, i.e., cv Sahrawy, cv Rihane/Lignee and the KSU bred lines. The cultivar Sahrawy withstands drought conditions and is suited for cultivation where water shortage prevails, it was also considered an early flowering cultivar (Morsy, 1979). However, in this study it required longer time to heading compared to other cultivars, it showed good vegetative growth and good number of grains. Al-Dos *et al.* (2000) recommended the use of this cultivar as green fodder plant and for grain production.

The RAPD results indicate that the level of RAPD polymorphism in the examined Saudi Arabian local and imported barley cultivars (13.25 bands per primer) is comparatively higher than the level of polymorphism revealed in Syrian barley cultivars, where only 3.83 bands per primer were recorded by other RAPD primers (Showman *et al.*, 2001), the Tibetan hulless barley varieties, where 9.6 bands per primer were reported (Yu *et al.*, 2002) and the European spring barley where Kuczynska *et al.* (2001) recorded 6.8 bands per primer. This may indicate that barley cultivars in Saudi Arabia are more diverse than the barley in Syria, the Tibet and Europe. However, the studied cultivars seem to be less diverse than the barley local populations in Sardinia, where five primers revealed 77 bands in 12 local populations (Papa *et al.*, 1995).

The analysis of RAPD profiles revealed some cultivar-specific bands that could be used to identify the different genotypes of barley cultivars; these include the presence of unique bands in cv Giza 123, cv Local cv Gustoe and cv Sahrawy and the absence of one band in cv Giza 121, cv Giza 123, cv Giza 128 and cv Sahrawy. Although cv Giza 123 and cv Gustoe are parents to Saudi bred cultivars, KSU 102, KSU 103 and KSU 104, none of the unique bands in the RAPD profile of the parent cultivars was evident in the profile of the Saudi bred cultivars These unique bands may define genetic finger printing that may be associated with one or more of the morphological traits. These unique bands may also prove useful for mapping of certain genes that may be associated with some features of the above-mentioned cultivars in future research. The cv Giza 123 produced the highest number of grains per spike, cv Gustoe is characterized by short stem, cv Giza 128 is characterized by a dropping spikes after ripening and cv Sahrawy by its tolerance to drought stress.

The morphological differences between the two-rowed cultivars and the six-rowed cultivars are reflected in the UPGMA tree and the NJ tree expressing the relationships of barley cultivars based on morphological data. Furthermore, the two-rowed cultivars were also separated from the six-rowed cultivars in the trees produced by the analysis of morphological and RAPD data combined. This result agrees with the results of several studies utilizing morphological and molecular evidence that have clearly supported the separation of the two-rowed and six-rowed cultivars of barley (Schut *et al.*, 1997; Koebner *et al.*, 2003; Komatsuda *et al.*, 1997). In the UPGMA and NJ trees based on RAPD data alone the two-rowed cultivars Local and Giza 128 were not separated together from the six-rowed cultivars. RAPD markers however, were reported to separate the two-rowed and six-rowed North American barley as two different groups (Hang *et al.*, 2000). In the present study RAPD markers provided evidence for the differentiation of closely related six-rowed cultivars e.g., cv Giza 123, cv KSU 102 and cv KSU 104, resembling the report by Hoffman *et al.* (1996) that RAPD markers enable differentiation of sex-rowed malting barley cultivars.

Both the UPGMA tree and the NJ tree, based on morphological criteria alone or in combination with RAPD data generally reflected the pedigree of some the studied cultivars. In particular, the UPGMA tree demonstrated the close relationships between the cultivars KSU 102 and KSU 104 developed by King Saud University and their parent cv Giza 123 (Table 1, Fig. 3 and 5). However, RAPD trees, are not in full agreement with the pedigree of some of the studied cultivars, for example the two cultivars Sahrawy and Rihane/Lignee appeared close to each other in spite of their morphological differences; similarly the two unrelated cultivars Giza 121 and KSU 102 appeared together in one group. On the other hand, the cultivars KSU 102 and KSU 104 developed by King Saud University were not grouped with their parent cv Giza 123. These findings are not in agreement with the reports by Tinker *et al.* (1993) and Fernândez *et al.* (2002) that RAPD fingerprinting may detect the pedigree of barley cultivars.

In conclusion, this study concluded that genetic relationships among the studied cultivars are expressed in their morphological traits and that RAPD data provide DNA fingerprinting that may be associated with certain traits. In addition, the results revealed unique markers that may help in identifying some cultivars of barley.

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